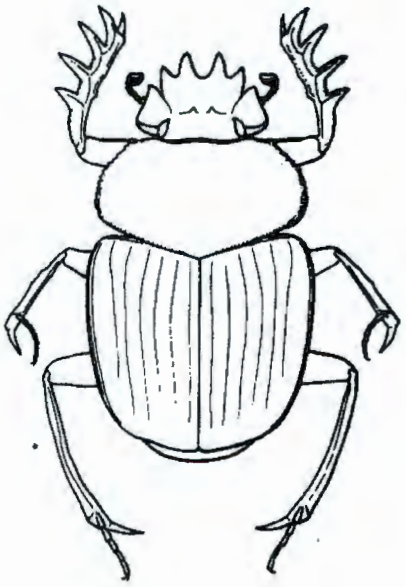
# Dung Beetles Eat Plants!

## Insights into the Nutritional World of

## *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeinae)



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Submitted in partial fulfilment of the degree of Bachelor of Science with Honours at
The University of Cape Town

2007

KD YATE

This thesis was supervised by Jeremy Midgley and Marcus Byrne.

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Megan Yates

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**Abstract**: Dung beetle eggs develop within the finite nutritional environment of the brood ball, which is made using maternally processed animal faeces. It is thought that microbial and gut-derived excretions constitute the major source of N and C for adult dung beetles, while developing larvae, which have retained the mouthparts of their saprophagous ancestors, digest larger particles in the brood ball and rely on symbionts present in the brood ball to provide breakdown products for their nutrition. Stable isotope analysis was used to trace the source of developing larval N and C. Nitrogen and carbon contents, as well as C: N ratios, were used to assess the nutritional quality of this finite food source and to track the changes in these values during the course of development. The main source of both larval and adult N and C was plant-derived and preferential assimilation of gut-derived excretions present in the dung did not occur. Symbionts, including fungi, did not appear to play a significant role in larval nutrition. Extensive amino acid recycling occurs during metamorphosis, indicated by the 0.53 ‰ enrichment in $\delta^{15}N$ in emergent beetles. Maternal processing of bulk dung creates an enhanced nutritional environment for offspring and the maternal faecal deposit, on which the egg is positioned, provides the larvae with an initial, nutrient-rich source of food.

**Keywords:** *Euoniticellus intermedius*, nutrition, carbon, nitrogen, isotopes

## Introduction

### *The Importance of Dung Beetles*

Dung beetles are soil-dwelling insects that rely on herbivore and omnivore dung both as a food source as well as for reproduction (Cambefort 1991). They are responsible for the processing, movement and consumption of vast quantities of mammal faeces and are highly important in natural and agricultural ecosystems (Bornemissza 1976). Dung beetles are key players in the world of nutrient cycling and provide valuable ecosystem services (Halffter & Edmonds, 1982). Indirect effects of dung beetle activity include suppression of dung-associated pests and pathogens and improvement of soil quality through aeration and water percolation resulting from the burrowing of paracoprid dung beetles (Bornemissza 1976). In the USA and Australia, *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeinae) has been introduced as an effective fly control agent. *Euoniticellus intermedius* is a paracoprid beetle that builds reticulated compound nests beneath the dung pat and has remarkably high fecundity (Cambefort & Hanski 1991).

### *Dung Beetle Life History Strategy*

Maternally processed dung is used to construct hollow spheres, known as brood balls, into which a single egg is laid (Halffter & Edmonds 1982, Heinrich 1991). Female beetles are responsible for digging the tunnels and making the brood balls, while males aid in the process by collecting dung from the surface and defending the nest (Heinrich 1991). Larvae live off the brood ball dung until metamorphosis and the nutrition available to the scarab larva is limited to the boundaries of the brood ball (Halffter & Edmonds, 1982). Food stress leads to plasticity in body size and a range of size associated traits (Emlen 1997). Premature exhaustion of larval food supply may trigger the initiation of metamorphosis (Shafiei *et al.* 2001).

### *Size Matters: The Importance of Nutrition*

Adult body size is determined by the nutritional environment available to the larva (Emlen, 1994, 1997, Moczek 1998, Halffter & Edmonds 1982, Hunt & Simmons 2000, 2002) and correlates positively with fitness and secondary sexual characteristics, such as horn size (Emlen 1997). Body size is a trait with low heritability; however the level of parental care affects the progeny's body size and fitness (Hunt & Simmons 2002). Larger beetles are able to roll larger balls and thus provide greater nutritional provisions for their offspring, making the level of care provided dependent on parent size (Hunt & Simmons 2002).

*Dung as a Food Source*

Dung is a patchily distributed ephemeral resource that provides a suitable habitat for a variety of organisms (Hanski 1987). Organisms exploiting this finite resource range from microorganisms and nematodes to a vast number of arthropods (Halffter & Edmonds 1982, Hanski 1991). Dung contains undigested food material, residues from the digestive tract as well as products of the gut flora and is considered to be nutrient-rich (Hanski 1987), even though much of the available protein and carbohydrates in the mammals' food source are absorbed during passage through the gut (Van Soest 1994). Dung has a high moisture content, ranging from 60 to 90%, and a pH between 6.5 and 8.0 (Hanski 1987). Seasonal variability of dung, which is influenced by the condition of the pasture on which the animals feed, has been shown to affect reproductive rate of *E. intermedius* (Edwards 1991). Dung can be divided into two distinct food sources; a nutrient-rich liquid component and a cellulose-rich solid component consisting mainly of undigested plant material.

Adult dung beetles feed on fine particulate matter in the dung while larvae, which have retained the mouthparts of their saprophagous ancestors, feed on larger particles of the dung (Holter *et al.* 2002). In adult dung beetles, coarse indigestible particles are rejected by filtering setae and the remaining small particles are concentrated on the molars by squeezing, which eliminates superfluous water (Holter 2000, Holter *et al.* 2002, Holter & Scholtz 2007 *In Press*). Selective feeding by typical dung beetles confines ingestion to particles with an MDIP (maximum diameter of ingested particles) of 20 μm (Holter & Scholtz 2007 *In Press*). MDIP shows some dependence on body size but, overall, coprophagous dung beetles restrict digestion to very small particles (Holter & Scholtz 2007 *In Press*). The larger particles mainly consist of cellulose-rich plant fibres too difficult for mammalian herbivores and dung beetles to digest (Holter & Scholtz 2007 *In Press*). The fine particles are thought to include easily digestible bacteria and epithelial cells derived from the herbivores gut (Holter & Scholtz 2007 *In Press*). Selective feeding lowers the C: N ratio from 13-39 in bulk dung to 7.3-12.6 (Holter & Scholtz 2007 *In Press*). The 20 μm fraction consumed has a higher nitrogen concentration compared to that of bulk dung (Holter & Scholtz 2007 *In Press*). Nitrogen concentrations of bulk dung ranged between 1 and 3.5% for a variety of mammalian herbivores, including elephants, zebra and wildebeest (Holter & Scholtz 2007 *In Press*). Holter & Scholtz (2007 In Press) suggest that most dung nitrogen is located in easily digestible gut-derived microbial biomass and epithelial cells and is assimilable, while ingested carbon is not. Since these beetles have no specialization,

such as enteric symbiotic associations, for the breakdown of complex plant structural carbohydrates, it is thought that the main source of carbon is the same as that of nitrogen i.e. fine mammalian gut-derived particles (Holter & Scholtz 2007 *In Press*). The larvae, however, have retained the mouthparts of their saprophagous ancestors and are unable to filter out the large indigestible particles (Holter 2000). Larval mouthparts allow ingestion of larger particles, which are macerated (Chown & Nicolson 2004).

*The Role of Symbionts in Dung Beetle Nutrition*

Polysaccharides, such as cellulose, hemicellulose and pectin, present in dung are digestible by microbial fermentation while the lignin in plant cell walls is indigestible (Van Soest 1994). Animals dependent on cellulose-rich food sources usually rely on symbiotic associations with microbes for cellulose digestion (Dow 1986, Martin 1991). The ability to digest cellulose is rare in insects; however certain insects rely on cellulose digestion by symbionts (Martin 1991). Endosymbiotic-containing insects include the American cockroach, *Periplaneta americana* (Cruden & Markovetz 1979), termites (Martin & Martin 1978) and the rhinoceros beetle, *Orycetes nasicornis* (Kukor & Martin 1987). Alternately, food may be microbially conditioned outside of the insect body, such as in attine ants, ambrosia beetles and fungus-growing termites, which cultivate fungal symbionts on a substrate and subsequently consume it (Kukor & Martin 1987). Siricid wood wasps (*Sirex cyanens*) and Cerambicid beetle larvae feed on wood-growing fungus (Kukor & Martin 1986). There are two classes of dung beetle-associated microsymbionts; cellulosobionts and coprobionts (Giodanich & Malan 1962). Cellulosobionts include anaerobic cellulose-degrading clostridia while coprobionts include all other saprophytic dung-associated microbes (Giodanich & Malan 1962). The assumption, based on mouthpart morphology, is that scarab larvae require enteric colonisation by cellulosobionts to provide cellulose breakdown products for their nutrition (Chown and Nicolson 2004). The larvae of some scarab species, such as *E. intermedius*, do not have a midgut fermenting chamber, which is used by cellulosobiont-reliant larvae, but rather have an undifferentiated intestine in which enteric cellulosobionts cannot establish (Halffter & Edmonds 1982). Larvae lacking a midgut fermentation chamber are thought to rely on coprobionts associated with the dung to provide cellulose-derived break-down products (Halffter & Edmonds 1982). These larvae are allegedly more closely associated with coprobionts and are more reliant on fresh, moisture-rich dung (Cambefort 1991). Mycophagy of fungi present in the brood ball may

play an important role in larval nutrition (Watkins 2005) since fungi are able to efficiently degrade lignin, a complex plant structural carbohydrate.

*The Faecal Deposit*

When female dung beetles lay an egg inside the brood ball, the egg is placed in an upright position using maternal faeces for support. Previous studies have labelled this a "maternal gift", which is thought to provide the larva with easily assimilable, nutrient-rich food (Watkins 2005, Maritz 2006). The faecal deposit does not affect development time or body size but it does seem to influence larval mortality (Watkins 2005). Symbiotic microorganisms present in the faecal deposit are thought to play a negligible role in larval development (Watkins 2005).

*The Application of Stable Isotopes to Nutritional Ecology*

Stable isotopes are a tool widely used for studying the nutritional ecology of a diverse range of organisms. They can be used to trace or identify food sources based on the assumption that the amount of $^{13}C$ in the body of an animal is generally similar to that in its diet (De Niro & Epstein 1978). Nitrogen isotopes have been used by ecologists to determine trophic behaviour in a wide variety of animals in aquatic and terrestrial systems, from large vertebrates to tropical termites. Enrichment in $\delta^{15}N$ of 3-4‰ occurs during the transfer of biomass from lower to higher trophic levels (De Niro & Epstein 1981, Mingwana & Wada 1984). An organism's isotope value depends on the value of its source nitrogen as well as isotopic discrimination and/or differential assimilation during food digestion and absorption and isotopic discrimination associated with discharge of excretion products (Olive *et al.* 2003). The fractionation step of trophic level enrichment is thought to occur during amino acid synthesis, which results in the excretion of the lighter $^{14}N$ isotope and retention of the heavier $^{15}N$ isotope (DeNiro & Epstein 1981, Gaebler *et al.* 1966, Gu *et al.* 1994, Minawaga & Wada 1984). Sponheimer *et al.* 2003 suggest that certain caution should be taken when using nitrogen isotope compositions as trophic level indicators because the enrichment trends are not always consistent and the actual fractionation steps leading to the observed enrichment can be complex.

Stable isotope ecology has also been applied, although not extensively, to insects. Nitrogen and carbon stable isotopes have been used to determine the diet of different termite species with different trophic habits (Tayasu *et al.* 1997). Elevated $\delta^{15}N$ values (8 to 16‰) have

been reported in adult raspberry beetles (*Byturus tomentosus*) collected shortly after emergence from over-wintering sites as well as in over-wintered larvae (11 to 14‰) (Scrimgeour *et al.* 1995). However, young adults collected after metamorphosis in autumn and larvae collected from raspberry fruit in summer did not show elevated $\delta^{15}N$ (Scrimgeour *et al.* 1995). Feeding larvae had $\delta^{15}N$ values similar (~2‰) to red raspberry (*Rubus idaeus*), their food plant (Scrimgeour *et al.* 1995). It is thought that extensive amino acid recycling during prolonged fasting leads to the observed elevated $\delta^{15}N$ values of overwintered larvae and adult raspberry beetles (Scrimgeour *et al.* 1995). A trophic level increase of 1‰ has been reported for the predatory seven-spot and two-spot ladybirds (*Coccinella septempunctata* and *Adalia bipunctata*) relative to their prey, the large raspberry aphid (*Amphorophora idaei*) (Scrimgeour *et al.* 1995). $\delta^{13}C$ values of chironomid larvae increased significantly throughout starvation, suggesting that chironomids preferentially break down components with lower, more depleted $\delta^{13}C$ values (Doi *et al.* 2007). $^{15}N$ enrichment during pupation has been observed in chironomids (Doi *et al.* 2007). In a tropical rainforest in North Queensland, Australia, stable isotope ratios of fifty ant species were obtained to determine the extent of plant derived resources vs. predation in their diet (Blüthgen *et al.* 2003). Variability in $\delta^{15}N$ between ant species was consistent with the trophic level increase predicted from feeding observations (Blüthgen *et al.* 2003). Stable isotope analysis has recently been used to determine the position of ants, spiders and other dominant invertebrate groups in a terrestrial food web (Sanders & Platner 2007). Stable isotope studies have therefore been successfully applied to insect communities and can provide insight into insect nutritional ecology.

In this study, stable isotope analysis was used to assess the nutritional environment available to *E. intermedius* during development as well as to determine the source, either plant or mammalian in origin, of larval and adult dung beetle N and C. Herbivores assimilate most of the easily available N and C in the consumed plant material (Van Soest 1994), and the source of N and C available to adult dung beetles is thought to be either in the form of microbial biomass and intestinal waste products, such as mucus, enzymes and dead epithelial cells (Holter & Scholtz 2007 *In Press*) or undigested plant material made available through coprobionts (Halffter & Edmonds 1982). Larvae, which have retained the mouthparts of their saprophagous ancestors, possibly rely on fungi for the breakdown of cellulose (Watkins 2005), are closely associated with coprobionts in the dung (Cambefort 1991) or assimilate gut-derived waste products present in the brood ball. These

assumptions are often made; however, no study has actually confirmed any of these postulations.

Questions to be answered:

1) Is the source of larval and adult N and C plant or mammalian in origin?
2) Do larvae rely on coprobionts or fungi present in the brood ball for cellulose breakdown?
3) Is the trophic level enrichment of $\delta^{15}N$ from dung to beetles altered by the presence of symbionts?
4) Does the maternal faecal deposit significantly contribute to larval nutrition?
5) Does maternal processing of bulk dung influence the nutritional environment of developing larvae?

To answer these questions, we analyzed brood ball and developing *E. intermedius* C and N contents and stable C and N isotope ratios during the course of development and compared these values to those of bulk dung, forage and cow hair. If larvae feed on basidiomycetes present in the dung, we would expect them to be 4‰ enriched in $^{13}C$ relative to the substrate (Gleixner *et al.* 1993, Kohzu *et al.* 1999). The presence of symbionts is expected to alter trophic level enrichment of $^{15}N$. If assimilation of gut-derived N occurs, we would expect a highly enriched $\delta^{15}N$ value in the larvae. The $\delta^{13}C$ value in the tissues is expected to be similar to that of the source – either plant or mammal – and to reveal the origin of C nutrition.

**Materials and Methods**

*Rearing of Dung Beetles*

A culture of *Euoniticellus intermedius* was derived from over 300 beetles collected from Cluny Farm, Greater Johannesburg, in 2007. Adult beetles were set in 1 L containers and were provisioned with fresh dung on compact, moist soil (as per Emlen 1997 and Moczek 1998). Dung in excess of the beetles' requirements was provided to prevent limitation of brood ball production. Dung, from cows that fed only on pasture, was collected from Dreyersdal Farm, Bergvliet, Cape Town and was frozen at -20°C after collection. Dung was defrosted 24 hours before use. Containers were kept in an insectary set at 25°C and 50% relative humidity, with a photocycle of LD 12:12 h. Up to 10 adult beetles were kept in each container, with the ratio of males to females being 2:3. *Euoniticellus intermedius*

were found to begin brood ball construction one to two days after setting. Four days after setting, adult beetles were removed from the containers and the brood balls were separated from the soil using a 3.00 mm sieve. Harvested brood balls were then placed in moist compact soil and allowed to develop. At different stages of development (egg-stage (initial), larval stage, pupal stage and just prior to adult beetle (final)), 10 brood balls were harvested oven-dried and weighed. Egg, larval head, pupae and beetle samples (10 of each) were collected, oven-dried and weighed. Grass and cow hair samples (5 of each) were obtained from Dreyersdal Farm, Bergvliet, Cape Town.

*Mass spectrometer determination*

Oven-dried grass, dung, brood ball, larvae, pupae, beetle and cow hair components were ground and weighed into 8 by 5 mm tin capsules (Elemental Microanalysis Ltd., Devon, U.K.) on a Sartorius microbalance (Goettingen, Germany). The samples were combusted in a Thermo Flash EA 1112 series elemental analyzer (Thermo Electron Corporation, Milan, Italy). The gases released were fed into a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy) via a Thermo Finnigan Conflo III control unit (Thermo Electron Corporation, Milan, Italy), where their $\delta^{13}C$ and $\delta^{15}N$ values were determined. This was done at the University of Cape Town, where our own internal standards were run to calibrate our results relative to international standards, $N_2$ in the air or $CO_2$ in PeeDee belemnite. The deviation of the sample from the international standard is expressed as $\delta^{13}C$, $\delta^{15}N = (R_{sample}/R_{standard}-1)*10^3$, where $R_{sample}$ represents the ratio between the heavy and lighter isotopes of the sample and $R_{standard}$ the ratio of the international standard.

*Statistical Analysis*

*Post-hoc* Tukey Least Significant Difference (LSD) tests were conducted to determine differences between samples.

**Results**

*Nitrogen Contents*

Nitrogen contents of grass, dung and brood balls were low, ranging from 1.63 to 2.61% and did not differ significantly ($p > 0.05$) (Fig. 1). The maternal faecal deposit, on which the egg is placed, had a nitrogen content of 3.67 ± 0.024 %, which was significantly higher than that of initial, larval, pupal and final brood balls ($p = 0.0045$ for initial balls), although

this was not significantly higher than bulk dung ($p = 0.3111$). Eggs were high in nitrogen, with a nitrogen content of 11.56%. Larvae, pupae and beetles had higher N contents; 9.92% in larvae, reaching 11.39% in emergent beetles. The nitrogen content of emergent beetles was significantly higher than that of larvae ($p = 0.0001$).

Brood balls contained a total of 13.23 ± 2.00 mg of N at the start of development and declined to 10.35±0.62 mg of N at the time of beetle emergence (Table 1). Thus, 2.88 mg of N from the brood balls was used during the course of growth and metamorphosis. Pupae contained 1.57±0.17 mg of N, 24.92% of which was lost during metamorphosis, with emergent beetles containing a total of only 1.18 ± 0.10 mg of N. Of the total amount of N present in the initial brood balls and eggs at the start of development, 10.31% could not be accounted for at the end, in balls harvested just prior to emergence and in pupae.

*Stable Nitrogen Isotope Ratios*

Cow dung was 1.58 ‰ depleted in $^{15}N$ relative to grass (Fig. 2). Cow dung is usually enriched by 2 ‰ compared to grass (Steele & Daniel 1978). Lupins, which are nitrogen-fixing legumes, were present in the pasture and may have formed part of the cows' diet, although the proportion was unknown. This may explain the inconsistency in $\delta^{15}N$ values between cow dung and grass. Since the relative contributions of $C_3$ grass and legumes were unknown, it may be more appropriate to rather predict a $\delta^{15}N$ value for forage of 3.81‰, based on the known enrichment of faeces relative to forage (Steele & Daniel 1978). Cow hair was 1.62 ‰ enriched in the heavier isotope relative to diet and is inconsistent with literature on diet-hair fractionation in cows, which is usually between 4 and 6.5 ‰ (Sponheimer *et al.* 2003). However, if we use the predicted forage value of 3.81 ‰, diet-hair fractionation is approximately 5.21 ‰, as expected.

Initial brood balls had a $\delta^{15}N$ value similar to that of dung ($p = 0.9348$). If we take a forage value of approximately 3.81 ‰, larval balls had a similar value of 3.63 ‰, having been depleted from the initial brood ball by 1.81 ‰. Pupal balls were significantly higher ($p = 0.0089$), enriched by 1.14‰ from larval balls. The faecal deposit did not differ significantly from the dung in $\delta^{15}N$ ($p = 0.0686$). The eggs, which were laid by adult beetles feeding on the cow dung, were 2.72 ‰ enriched, compared to dung $\delta^{15}N$. $\delta^{15}N$ declines from the eggs to the larvae. There is a fractionation event during metamorphosis, with the emergent beetles being 0.53 ‰ enriched compared to the pupae. There was a 2.07

‰ increase in $\delta^{15}N$ between initial brood balls and pupae, which represents the trophic level fractionation.

*Carbon Content*

The percent carbon in the initial brood ball was 13.99% lower than in the cow dung (Fig. 3) ($p = 0.0003$). The percentage of C in the brood balls decreased from initial balls to larval and pupal balls and then declined further in balls harvested just prior to beetle emergence, while the percentage of C in growing *E. intermedius* increased.

The total carbon content of emergent beetles was 3.51% (or 2.57mg) lower than that of pupae, with changes during metamorphosis requiring 35.71% of the total C present in the pupae (Table 2). Approximately 29.74% (59.05 mg) of the total C present in eggs and brood balls at the start could not being accounted for at the end (in final balls and pupae) and was presumably lost as $CO_2$ from respiration.

*Carbon Isotopes*

Cow hair had a $\delta^{13}C$ value, of -16.08 ± 0.61 ‰, which was highly enriched compared to grass (Fig. 4). Diet-hair fractionationation for cows is usually +3.2 ‰ (Sponheimer *et al.* 2003). Since the proportions of grass and legumes in the diet were unknown, we predicted a forage $\delta^{13}C$ value of -19.23 ‰ based on this known diet-hair fractionation value. Initial brood balls had a $\delta^{13}C$ value similar to that of dung ($p = 1.000$). $\delta^{13}C$ values of the brood balls did not change significantly during the course of larval development and metamorphosis. Eggs had a $\delta^{13}C$ value similar to that of cow dung ($p = 1.000$). $\delta^{13}C$ values of larvae, pupae and beetles were significantly enriched relative to cow dung ($p = 0.0177$ for larvae). During growth and metamorphosis, $\delta^{13}C$ increased, with emergent beetles being 2.39 ‰ enriched relative to initial brood balls ($p = 0.0001$). Emergent beetles had a $\delta^{13}C$ value similar (+ 0.02 ‰) to that of the predicted forage value of -19.23 ‰.

*C: N Ratios*

The C: N ratio of initial brood balls was significantly lower than that of bulk dung ($p = 0.0003$) (Fig. 5). The C: N ratios of brood balls declined throughout development, while it remained very low in eggs and developing scarabs throughout the course of development. The maternal faecal deposit had a C: N ratio of 10.49±0.07, which was significantly lower than that of dung and initial brood balls ($p = 0.0001$ and $p = 0.0001$ respectively).

**Discussion**

*Where do dung beetles get their N from?*

The $\delta^{15}N$ value of forage was difficult to assess because the proportions of grass and legume in the cow dung were unknown. Cow dung was depleted in $^{15}N$ relative to grass, whereas it should have been enriched by 2 ‰ (Steele & Daniel 1978). Therefore a predicted forage value of 3.81 ‰ was used based on the value in the cow dung. Larvae were 2.14 and 3.95 ‰ enriched relative to initial and larval brood balls, respectively, consistent with a trophic level increase in $\delta^{15}N$ (De Niro & Epstein 1981) (Fig. 2). In general, enrichment in $\delta^{15}N$ of 3-4 ‰ occurs during the transfer of biomass from lower to higher trophic levels (De Niro & Epstein, Minawaga & Wada 1984). This low degree of trophic level enrichment is suggestive of herbivory. If developing beetles were selectively feeding on a mammalian-derived N source, they would essentially be in the third trophic level and this would be reflected in their $\delta^{15}N$ value, which would be expected to be 6-8 ‰ higher than that of forage. Since the brood ball is made from dung, it consists of undigested forage material as well as gut-derived microbial biomass and epithelial cells. The $\delta^{15}N$ value of gut-derived components was not assessed, however, if we use the predicted forage value of 3.81 ‰, larvae were 3.77 ‰ enriched relative to forage. If larvae were feeding on gut-derived products, they would be considered to be in the third trophic level and the enrichment in larvae would be substantially higher than the one trophic level increase of 3.77 ‰ observed. For termites, a wide range of isotopic effects (-1.6 to + 8.8 ‰) from diet to termite tissue has been observed (Tayasu *et al.* 1997). A trophic level increase of 1 ‰ has been reported for the predatory seven-spot and two-spot ladybirds (*Coccinella septempunctata* and *Adalia bipunctata*) relative to their prey, the large raspberry aphid (*Amphorophora idaei*) (Scrimgeour *et al.* 1995). Since trophic level enrichment has not been extensively studied in scarabs, the enrichment may be lower or higher than it is for other animals and may significantly alter the interpretation of these results.

The maternal faecal deposit had a similar $\delta^{15}N$ value to cow dung (Fig. 2). Thus, during passage through the gut of adult beetles, the N isotopic signal of food material remained unchanged. This may imply that adult dung beetles do not preferentially assimilate the compounds containing the heavier isotope, such as cow epithelial cells, in which case we would expect the faecal value to decline to a value closer to that of forage. It has previously been presumed that gut-derived excretions, including epithelial cells, mucus and

microbial biomass, were assimilated by adult dung beetles (Holter & Scholtz 2007 *In Press*). This is the first study to actually test these widely spread assumptions and confirm that dung beetles do not in fact selectively assimilate gut-derived excretions from the dung. Larval balls had a $\delta^{15}N$ value similar to that of the predicted forage value, having been depleted by 1.81 ‰ from initial brood balls. This may indicate that nitrogen derived from a source with a higher $\delta^{15}N$ value than that of forage is assimilated by the larvae from the brood ball or that the lighter isotope gets excreted, and deposited in the ball, during deamination. Eggs were enriched in $^{15}N$, possibly due to fractionation events occurring during synthesis.

*What is the source of dung beetle C?*

An organism's isotope value depends on the value of its source nitrogen as well as isotopic discrimination and/or differential assimilation during food digestion and absorption and isotopic discrimination associated with discharge of excretion products (Olive *et al.* 2003). Cow hair had a $\delta^{13}C$ value, of -16.08 ± 0.61 ‰, which was highly enriched compared to grass (Fig. 4). Diet-hair fractionationation is usually +3.2 ‰ (Sponheimer *et al.* 2003) and since the proportions of grass and legumes in the diet were unknown, we predicted a forage $\delta^{13}C$ value of -19.23 ‰ based on this known diet-hair fractionation value. $\delta^{13}C$ values of larvae, pupae and beetles were significantly higher than that of cow dung. There was no concurrent decrease in $\delta^{13}C$ in brood balls through development. The progressive increase in beetle $\delta^{13}C$ throughout development, with no simultaneous decline in brood ball $\delta^{13}C$, suggests a fractionation event during metabolism, with the lighter $^{12}C$ isotope lost as $CO_2$. If selective assimilation of mammal-derived C occurred, then we would expect a change in $\delta^{13}C$ of brood balls. This implies that developing larvae feed on plant-derived C present in the dung. The eggs had a $\delta^{13}C$ value similar to cow dung and the predicted forage value, which would indicate that adult beetles also feed on plant-derived C in the dung. If they were in fact feeding on ruminant-derived epithelial cells we would expect eggs to have an enriched signal relative to forage. The faecal deposit also had a $\delta^{13}C$ value similar to that of cow dung. If adult beetles were selectively assimilating C from gut-derived excretions, the maternal faecal deposit would have a depleted signal.

Dung beetles fall into a small number of families in the superfamily Scarabaeoidae, section Laparosticti (Cambefort 1991). The majority of this family are saprophagous, both in the larval and adult stages and it is thought that dung beetle ancestors were saprophagous

(Cambefort 1991). However, saprophagy and coprophagy are not very different feeding habits. The switch from saprophagy to coprophagy is associated mainly with changes in mouthparts, from hard, biting mandibles to soft, filtering ones (Cambefort 1991). In adult Aphodiidae and Scarabaeidae, soft-filtering mandibles have evolved; however, some taxa are still saprophagous in the adult stage (Cambefort 1991). The soft, filtering mouthparts of dung beetles confine ingestion to finer particles, effectively lowering the C: N ratio and increasing the nitrogen content of ingested food (Holter & Scholtz 2007 *In Press*). Some saprophagous beetles evolved specialized mandibles and feed on fine-grained humus which has the highest nutrient quality (Cambefort 1991). In the tropics, the high availability of mammalian dung is thought to have promoted the evolution of coprophagy from saprophagy (Cambefort 1991). Scarabaeidae which had already evolved mouthparts to effectively utilize the most nutrient-rich component of the humus were in the best position to take advantage of the dung resources (Cambefort 1991). Our results suggest that, in both the adult and larval stages, dung beetles feed on plant-derived N and C present in the dung. In coprophagous adult beetles, filterering occurs and finer particles are ingested (Holter & Scholtz 2007 *In Press*), however, the actual origin of N and C remains unchanged from that of saprophagous beetles. Thus, in the evolution of coprophagy from saprophagy, no other adaptations, such as selective assimilation, have occurred and coprophagy and saprophagy are not exceptionally different feeding habits.

*Do fungi play a role in larval nutrition?*

Basidiomycetes are generally 4‰ richer in $^{13}C$ than their substrate (Gleixner *et al.* 1993, Kohzu *et al.* 1999). If larvae were feeding on fungi present in the brood ball, we would expect a 4‰ increase in larval $\delta^{13}C$ relative to the substrate, which was not observed. Fungi therefore appear to play no significant role in the nutrition of larvae which have an undifferentiated intestine in which cellulosobionts cannot establish. Fungus-growing ants culture fungal symbionts on freshly cut leaves, plant debris and insect faeces and the fungal tissue constitutes the exclusive food of the ant larvae and a minor component of the adult workers (Kukor & Martin 1987). In termites (Macrotermitinae), stable carbon isotope analysis has been used to show the differential role of symbiotic fungi; in some species, fungi are used for lignin degradation while in others the fungi is consumed (Hyodo *et al.* 2003). The larvae of *E. intermedius* repeatedly consume the brood ball contents, which may increased the larval assimilation efficiency and enable them to develop independently of symbionts.

*Brood Ball Construction*

During brood ball construction, bulk dung is maternally processed and moulded into a hollow sphere into which a single egg is placed. Maternal processing results in brood balls with a significantly lower C content than dung (Fig. 3). The N content, however, remained unchanged (Fig. 1). Initial brood balls therefore had a C: N ratio considerably lower than that of dung. When adult dung beetles feed on bulk dung, coarse indigestible particles are rejected by filtering setae (Holter 2000, Holter *et al.* 2002). Our results suggest that this occurs during brood ball construction, with this material being used for brood ball manufacture rather than being eaten by the adult. This would suggest an additional function for filtering setae; that of constructing a suitable nutritional brood ball environment for offspring. This filtering effectively eliminates large indigestible plant components, consisting mainly of cellulose, from the brood ball, enhancing the amount of assimilable nutrition present for developing larvae. Stable carbon and nitrogen isotope ratios of newly-constructed brood balls did not differ significantly from those of bulk dung (Figs. 2 and 3). This indicates that dung-associated coprobionts do not alter the stable N and C isotopic signals in dung and may therefore not play a role in larval nutrition, consistent with the findings of Watkins (2005).

*The Role of the Faecal Deposit: A Maternal Gift?*

The faecal deposit was significantly higher in % N than initial brood balls (Fig. 1) and had a C: N ratio lower than that of bulk dung and initial brood balls (Fig. 5). This is in agreement with Holter & Scholtz (2007 *In Press*), who show that selective feeding occurs in adult dung beetles, confining ingestion to particles with an MDIP of 20µm, effectively rejecting large indigestible plant particles in the dung and lowering the C: N ratio to 7.3-12.6 from bulk dung (with a C: N ratio of bulk dung ranging between 13-39 for a variety of herbivores). Watkins (2005) concluded that the faecal deposit was a 'maternal gift', consisting of easily assimilated, nutrient-rich food which supplements larval nutrition in the early stages of development. Our results show that the maternal faecal deposit probably serves as an initial food source for larvae, in agreement with Watkins (2005).

*Metamorphosis: The Costs*

Of the total amounts of N and C present in pupae, high percentages (24.92% of total N and 35.71% of total C) were lost during metamorphosis (Tables 1 and 2). The cost of metamorphosis is thus high, with large amounts of C used for respiration to fund the

changes occurring during metamorphosis. Nitrogen may be lost when the exuvae gets shed during metamorphosis. $\delta^{15}N$ and $\delta^{13}C$ increased during metamorphosis, due to metabolic fractionation. Increases in $\delta^{15}N$ are indicative extensive amino acid recycling and also occur in overwintered larvae and adult raspberry beetles (Scrimgeour *et al.* 2003).

**Conclusion**

This is the first study to test the widely made, but as yet untested, assumptions regarding the origin of larval and adult N and C. Adult dung beetles as well as larvae derive their N and C from plant material present in the dung and brood balls and do not preferentially assimilate gut-derived excretions. This suggests that the coprophagous feeding habits employed by dung beetles do not differ significantly from the saprophagous feeding habits of their close relatives and ancestors. We suggest an additional role for filtering setae of adult beetles; that of processing bulk dung during brood ball construction, thereby enhancing the nutritional environment available to developing larvae. The maternal faecal deposit provides larvae with an initial nutrient-rich food source. Symbionts do not appear to play an important role in larval nutrition and may not be necessary due to the repeated feeding on brood ball contents by the larvae, which possibly increases larval assimilation efficiency. Metamorphosis incurs high costs in terms of N and C and extensive amino acid recycling occurs, resulting in emergent beetles that are enriched in $^{15}N$.

**Acknowledgements**

Funding assistance was provided by the University of Cape Town and the National Research Foundation (NRF). I would like to thank Jeremy for his enthusiasm and valuable, insightful advice regarding the nutrition of dung beetles. Marcus passed on his beetle-rearing skills as well as his passion for these fascinating creatures and offered constant support and thought-provoking questions to be explored throughout this project.

**References**

Blüthgen, N., Gebauer, G. and Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* **137**:426-435

Bornemissza, G.E. (1976) The Australian dung beetle project 1965-1975. *Aust. Meat Res. Comm. Rev* **30**: 1-30

Cambefort, Y. (1991) From saprophagy to coprophagy. In: Dung Beetle Ecology (Eds Hanski, I. and Cambefort, Y.) pp. 22-35. Priceton University Press, Princeton, New Jersey

Cambefort, Y. and Hanski, I. (1991) Dung Beetle Population Biology. In: Dung Beetle Ecology (Eds Hanski, I. and Cambefort, Y.) pp. 36-50. Priceton University Press, Princeton, New Jersey

Chown, S.L. and Nicolson, S.W. (2004) Insect Physiological Ecology. Oxford University Press, UK.

Cruden, D.L. and Markovetz, A.J. (1979) Carboxymethyl cellulose decomposition by intestinal bacteria of cockroaches. *Applied Environmental Microbiology* **38**: 369-372

De Niro, M.J. and Epstein. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**: 5

DeNiro M.J. and Epstein S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**: 341–351.

Doi, H., Kikuchi, E., Takagi, S and Shikano, S. (2007) Changes in carbon and nitrogen stable isotopes of chironomid larvae during growth, starvation and metamorphosis. *Rapid Communications in Mass Spectrometry* **21** (6): 997-1002

Dow (1986) Insect midgut function. *Advances in Insect Physiology* **19**: 188-290

Edwards, P.B. (1991) Seasonal variation in the dung of African grazing mammals, and its consequences for coprophagous insects. *Functional Ecology* **5**: 617-628

Emlen, D.J. (1994) Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae) *Proceedings of the Royal Society of London* **256**: 131-136

Emlen, D.J. (1997) Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society of London* **264**: 567-574

Gaebler, O.H., Vitti, T.G. and Vukmirovich, R. (1966) Isotope effects in metabolism of $^{14}N$ and $^{15}N$ from unlabelled dietary proteins. *Can. J. Biochem.* **44**: 1249-1257

Giodanich, A. and Malan, C.E. (1962) Sulla fonte di allimentazione e sulla microflora aerobica del nido pedotrofico e dell'apparato digerente delle larve di Scarabei coprofagi (Coleoptera: Scarabaeidae). Atti dell'Academia Scienze di Torino **96**: 575-628

Gleixner, G., Danier, H-J, Werner, R.A., Schmidt, H-L. (1993) Correlations between the $^{13}C$ content of primary and secondary plant products in different cells and that in decomposing basidiomycetes. *Plant Physiology* **102**: 1287-1290

Gu, B., Schell, D.M. and Alexander, V. (1994) Stable carbon and nitrogen isotopic analysis of the plankton food web in a subarctic lake. *Can. J. Fish. Aquat. Sci.* **51**: 1338-1344

Halffter, G. and Edmonds, W. (1982) The nesting behaviour of dung beetles (Scarabaeinae): an ecological and evolutive approach. Instituto de Ecologia, Mexico, DF.

Hanski, I. (1987) Nutritional Ecology of Dung- and Carrion-Feeding Insects. pp 837-884. In: Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates. (Eds: Slansky, F. and Rodriguez, J.G). John Wiley and Sons, Inc., US.

Heinrich, B. (1991) The ways of coprophiles. *Science* **254**: 5033: 873-874

Holter, P. (2000) Particle feeding in Aphodius dung beetle (Scarabaeidae): old hypotheses and new experimental evidence. *Functional Ecology* **14**: 631-637

Holter, P., Scholtz, C.H. and Wardaugh, K.G. (2002) Dung feeding in adult scarabaeines (tunnellers and endocoprids): even large dung beetles eat small particles. *Ecological Entomology* **27** (2): 169-176

Holter, P. and Scholtz, C.H. (2007) What do dung beetles eat? *In Press*

Hunt, J. and Simmons, L.W. (2000) Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. *Evolution* **54** (3): 936-941

Hunt, J. and Simmons, L.W. (2002) The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. *Proceedings of the National Academy of Science of the United States of America* **99** (10): 6828-6832

Hyodo, F., Tayasu, I., Inoue, T., Azuma, J.I., Kudo, T., and Abe, T. (2003) Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). *Functional Ecology* **17**: 186-193

Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K. and Wada, E. (1999) Natural 13C and 15N abundance of field collected fungi and their ecological implications. *New Phytologist* **144** (2): 323-330

Kukor, J.J. and Martin, M.M. (1987) Nutritional Ecology of Fungus-Feeding Arthropods. pp 791-814. In: Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates. (Eds: Slansky, F. and Rodriguez). J.G. John Wiley and Sons, Inc., US.

Maritz, M. (2006) The content of the maternal gift in Euoniticellus intermedius (Reiche) (Coleoptera: Scarabaeinae). Unpublished Honours dissertation. University of the Witwatersrand, Johannesburg, South Africa.

Martin, M.M. and Martin, J.S. (1978) Cellulose digestion in the midgut of the fungus-growing termite *Macrotermes natalensis*: the role of acquired digestive enzymes. *Science* **199**: 1453-1455

Martin, M.M. (1991) The evolution of cellulose digestion in insects. *Philosophical Transactions: Biological Sciences* **333** (1267): 281-287

Moczek, A.P. (1998) Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behavioural Ecology* **9**: 636-641

Minawaga, M. and Wada, E. (1984) Stepwise enrichment of $\delta^{15}N$ along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochim. Cosmochim. Acta* **48**: 1135-1140

Olive, P.J.W., Pinnegar, J.K., Polunin, N.V.C., Richards, G. and Welch, R. (2003) Isotope trophic-step fractionation: a dynamic equilibrium model. *Journal of Animal Ecology* **72**: 608-617

Sanders, D. and Platner, C. (2007) Intraguild interactions between spiders and ants and top-down control in a grassland food web. *Oecologia* **150**: 611-624

Shafiei, M., Moczek, A.P., and Nijhout, H.F. (2001) Food availability controls the onset of metamorphosis in the dung beetle, *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Physiological Entomology* **26**: 173-180

Scrimgeour, C.M., Gordon, S.C., Handley, L.L. and Woodford, J.A.T. (1995)Trophic Levels and Anomalous $\delta^{15}N$ of Insects on Raspberry (Rubus Idaeus L.) *Isotopes in Environmental and Health Science* **31** (1):107-115

Sponheimer, M., Robinson, T. Ayliffe, L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D. and Ehleringer, J. (2003) An experimental study of carbon-isotope fractionation between diet, hair and faeces of mammalian herbivores. *Can. J. Zool.* **81**:871-876

Sponheimer, M., Robinson, T, Ayliffe, L., Roeder, B., Hammer, J., Passey, B., West, A., Cerling, T., Dearing, D. and Ehleringer, J. (2003) Nitrogen Isotopes in Mammalian Herbivores: Hair $\delta^{15}N$ Values from a Controlled Feeding Study. *International Journal of Osteoarcheology* **13**: 80-87

Steele, K.W. and Daniel, R.M. (1978) Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of $^{15}N$ for tracer studies. *J. agric. Sci., Camb.* **90**: 7-9

Tayasu, I., Abe, T., Eggleton, P. and Bignell, D.E. (1997) Nitrogen and carbon isotope ratios in termites: an indicator of trophic habit along the gradient from wood-feeding to soil-feeding. *Ecological Entomology* **22**: 343-351

Van Soest, P. (1994) Nutritional ecology of the ruminant 2nd Edition. Cornell University Press, USA.

Watkins, B. (2005) Is the faecal deposit a microbial 'gift': The nature and mechanism of the role of the maternal faecal deposit in brood balls of *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeinae). Unpublished Honours dissertation. University of the Witwatersrand, Johannesburg, South Africa.

**Tables**

**Table 1.** Total nitrogen content (mg) (mean ± S.E.) and the percentage of the initial amount of total N present in *E. intermedius* brood balls at the start and end of development and in eggs, pupae and emergent beetles. The total initial amount of N present was taken as N in the brood balls as well as N in the eggs.

| | Total N (mg) | % of Initial N |
|---|---|---|
| Initial brood ball | 13.23±2.00 | 99.54 |
| Final brood ball | 10.35±0.62 | 77.88 |
| Egg | 0.06±0.002 | 0.46 |
| Pupae | 1.57±0.17 | 11.80 |
| Emergent beetle | 1.18±0.10 | 8.86 |

**Table 2.** Total carbon content (mg) (mean±S.E.) and the percentage of the initial amount of total C present in *E. intermedius* brood balls at the start and end of development and in eggs, pupae and emergent beetles. The initial amount of C present was taken as C in the brood balls as well as C in the eggs

| | Total C (mg) | % of Initial C |
|---|---|---|
| Initial brood ball | 198.31±38.10 | 99.88 |
| Final brood ball | 132.28±12.89 | 66.63 |
| Egg | 0.23±0.01 | 0.12 |
| Pupae | 7.21±0.73 | 3.63 |
| Emergent beetle | 4.64±0.61 | 2.33 |

**Figure Legends**

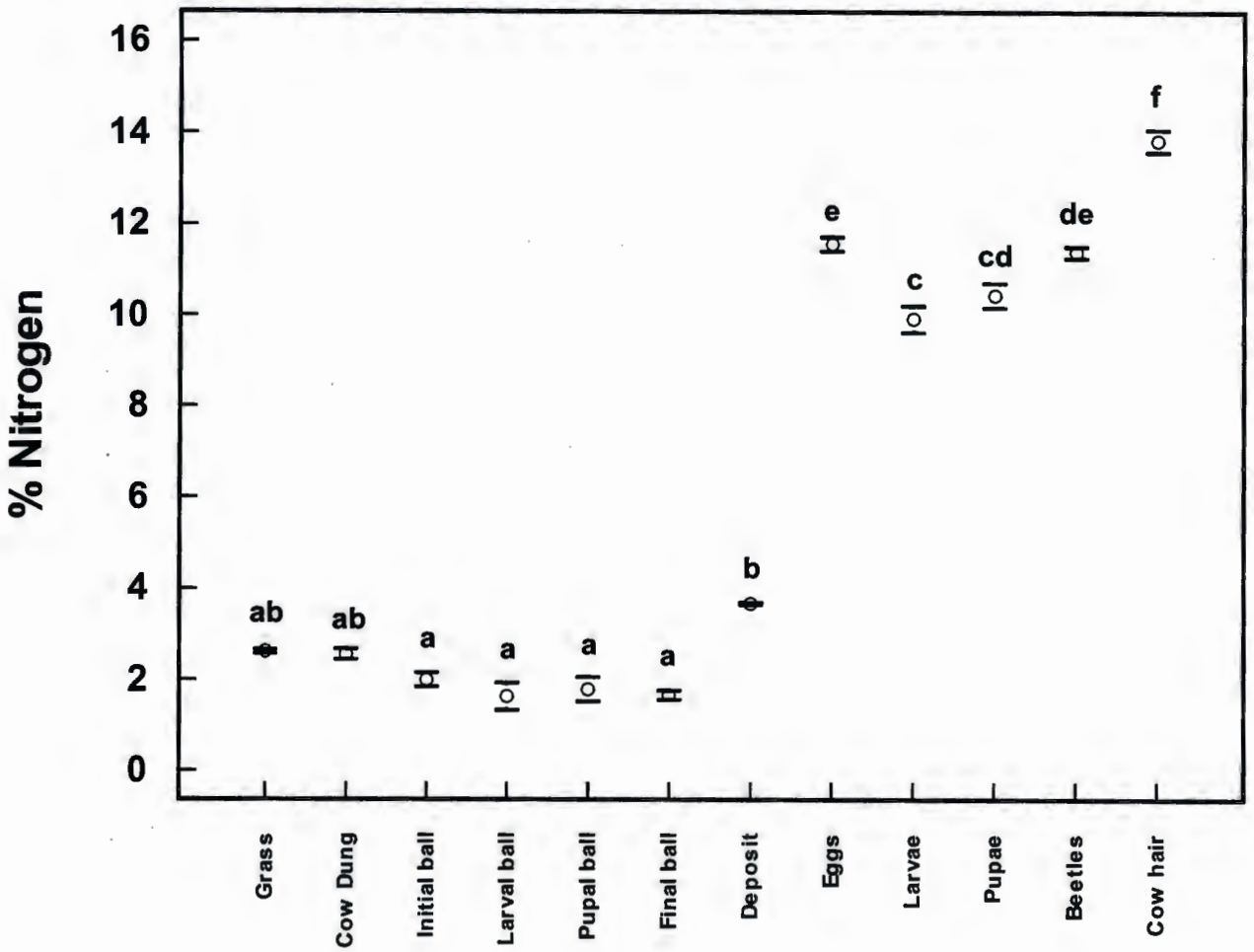
**Figure 1.** Nitrogen content of grass, dung, cow hair and brood balls and *E. intermedius* harvested at different times during development. Values represent means ± standard errors. Dissimilar letters above squares indicate significant differences ($P < 0.05$) in nitrogen content determined by one-way ANOVA *post-hoc* Tukey LSD tests. *E. intermedius* progressively increased in %N throughout development.

**Figure 2.** Stable nitrogen isotope ratios of grass, dung, cow hair and brood balls and *E. intermedius* harvested at certain times during growth. Values represent means ± standard errors. Different letters above squares show significant differences ($P < 0.05$) between the $\delta^{15}N$ values, which were determined using one-way ANOVA *post-hoc* Tukey LSD tests. There was a fractionation step during metamorphosis, with emergent beetles 0.53‰ more enriched than pupae, and significantly enriched relative to brood balls.

**Figure 3.** Carbon content of brood balls and *E. intermedius* harvested at different times of development as well as grass, dung and cow hair. Values represent means ± standard errors. Dissimilar letters above squares indicate significant differences ($P < 0.05$) in carbon content determined by one-way ANOVA *post-hoc* Tukey LSD tests. %C declined in brood balls and increased in growing *E. intermedius* during the course of development.

**Figure 4.** Stable carbon isotope ratios of brood balls and *E. intermedius* individuals harvested throughout development, as well as grass, bulk dung and cow hair. Values represent means ± standard errors. Different letters above squares indicate significant differences ($P < 0.05$) in $\delta^{13}C$ determined by one-way ANOVA *post-hoc* Tukey LSD tests. *E. intermedius* became progressively enriched in $^{13}C$ throughout development. $\delta^{13}C$ values of larvae, pupae and emergent beetles were significantly higher than brood ball and bulk dung values.

**Figure 5**. C: N Ratios of *E. intermedius* individuals and brood balls harvested at various stages of growth and metamorphosis in addition to grass, cow hair and bulk dung. Values represent means ± standard errors. Different letters above squares indicate significant differences ($P < 0.05$) in carbon content determined by one-way ANOVA *post-hoc* Tukey LSD tests. The C: N ratio of brood balls declines during the course of larval development and pupal development. C: N ratios of eggs, larvae, pupae and beetles were low and remained similar during growth and metamorphosis.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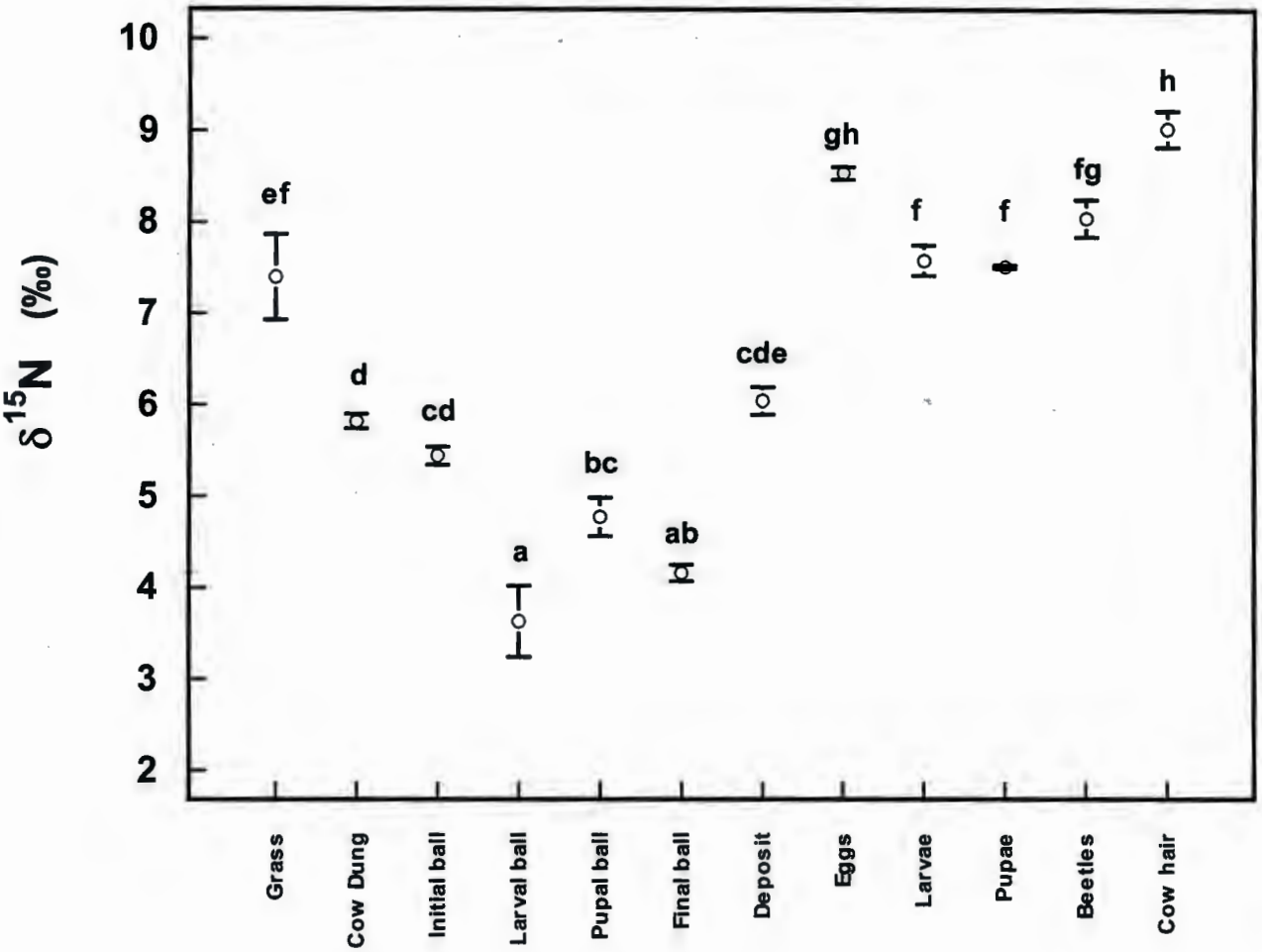
Pupae
Beetles
Cow hair

Fig. 1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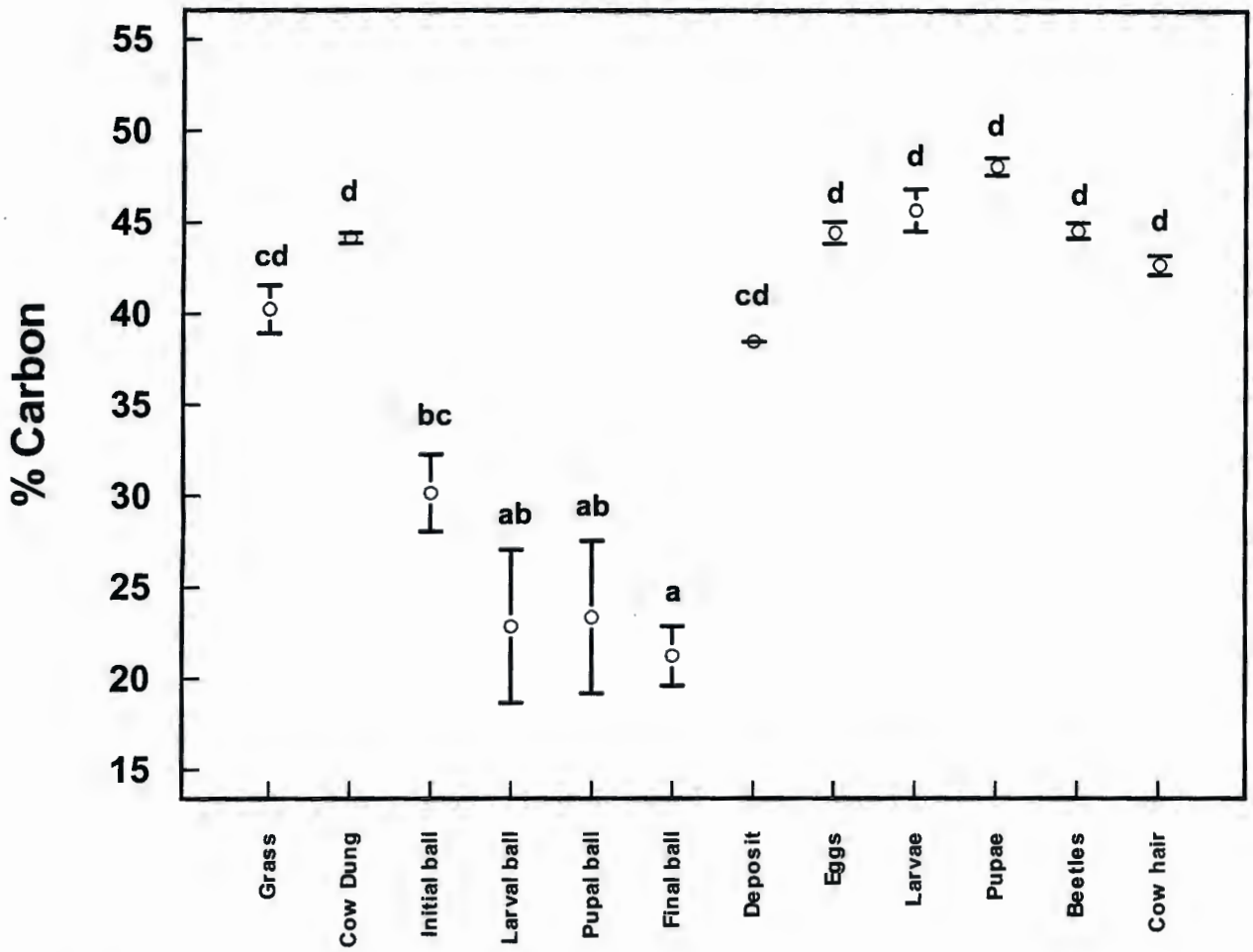
Beetles
Cow hair

Fig. 2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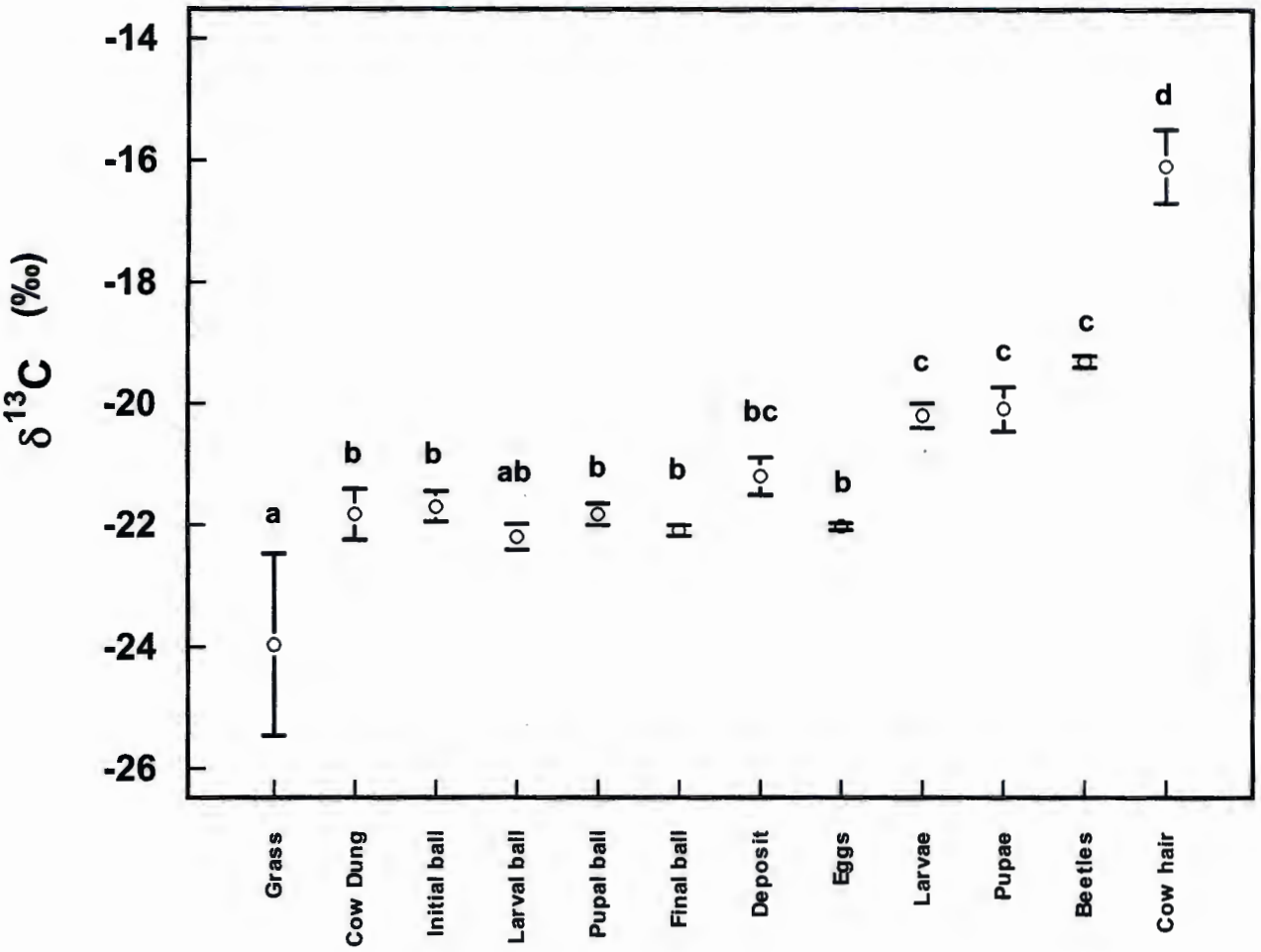
Cow hair

Fig. 3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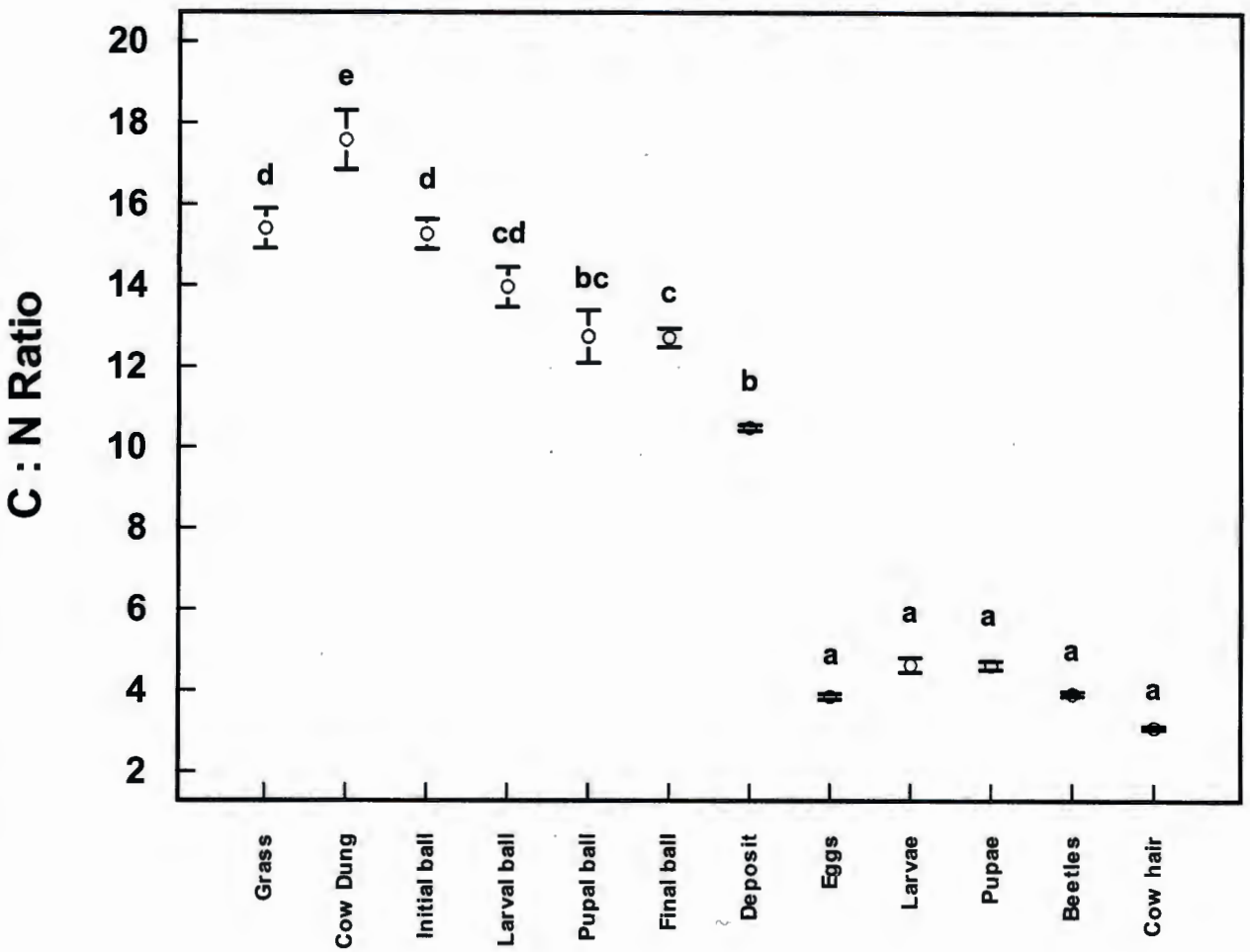
Beetles
Cow hair

Fig. 4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Grass
Cow Dung
Initial ball
Larval ball
Pupal ball
Final ball
Deposit
Eggs
Larvae
Pupae
Beetles
Cow hair

Fig. 5